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AFLP marker associations with agronomic and fiber traits in cotton

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Abstract DNA markers linked with major QTL contributing to traits of importance will be a useful tool for cotton (*Gossypium spp.*) genetics and breeding. We crossed four photoperiod-sensitive accessions of cotton, *G. hirsutum* L., with a cultivar, selected day-neutral plants and backcrossed four times to each of the four photoperiod-sensitive accessions, selecting day-neutral plants at each generation. The day-neutral plants from the first cross and the four backcross generations were advanced to the F_6. These 20 day-neutral lines and four cultivars were grown in two environments at Mississippi State, MS and scored for seven agronomic and fiber quality traits. They were also scored for AFLP markers using a bulk sample of leaves from each of 24 lines. More than 50 AFLP markers were associated with the seven traits with fewer markers associated with fiber than agronomic traits. However, one to four markers were associated with 22–93% of the phenotypic variability of each of the seven traits. The results suggest that selected markers could be used in marker assisted selection (MAS) in crosses designed to use alleles from exotic accessions or cultivars to develop elite breeding lines for cotton improvement.

Keywords AFLP · QTL · Cotton · Marker-assisted selection · Yield traits · Fiber traits

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Introduction

Identifying associations of molecular markers with quantitative traits is essential for marker-assisted selection (MAS) in plant genetics and breeding research. Its importance in cotton genetics and breeding programs has also been recognized since 1992 (Meredith 1992; Cantrell and Davis 1993; Paterson 1993; Reinisch et al. 1994). However, genetic polymorphism at the molecular level within *Gossypium hirsutum* L. is relatively low (Wendel et al. 1992; Tatineni et al. 1996; Jiang et al. 1998, 2000; Pilly and Myers 1999; Abdalla et al. 2001; Brubaker and Wendel 2001;

Gutierrez et al. 2002; Lu and Myers 2002; Jin 2003; Zhang et al. 2005). On the other hand, interspecific crosses have led to many genomic studies (Reinisch et al. 1994; Lazo et al. 1995; Saha et al. 1995; Paterson and Zhao 1995; Wright et al. 1998, 1999; Paterson et al. 2003; Lacape et al. 2003; Mei et al. 2004; Rong et al. 2004; He et al. 2005; Lacape and Nguyen 2005). With the use of aneuploid substitution stocks, some individual RFLP, AFLP, and SSR markers have been assigned to specific chromosomes (Liu et al. 2000a, b; Lacape et al. 2003; Rong et al. 2004; Ulloa et al. 2005). Two linkage maps covering more than 80% of the genome were both from *Gossypium hirsutum* × *Gossypium barbadense* (Lacape et al. 2003; Rong et al. 2004).

The construction of linkage maps has led to mapping QTLs contributing to traits of interest from populations of *G. hirsutum* (Shappley et al. 1998a, b; Ulloa and Meredith 2000; Zhang et al. 2001, 2003; Guo et al. 2003; Zuo et al. 2000; Ulloa et al. 2002, 2005; Zhang et al. 2005) and from interspecific populations (Jiang et al. 1998, 2000; Ulloa et al. 2000; Kohel et al. 2001; Paterson et al. 2003; Mei et al. 2004; He et al. 2005; Lacape et al. 2005; Lin et al. 2005; Shen et al. 2005, 2006). Most of the above studies showed that genetic control of agronomic and fiber traits was highly complex, translating into high numbers of QTL and moderate to low additive effects (Lacape et al. 2005). On the other hand, comparisons between experiments and/or populations have been difficult and limited because few common markers were detected among different populations. These factors indicate that the use of MAS for cotton enhancement may be impractical at this time.

The narrow genetic base of the upland cotton germplasm that is used in breeding programs is considered one of the contributing factors in the lack of major progress in the improvement of yield and fiber properties in U.S. cultivars over the last 15 years, (Meredith 2000; Lewis 2001). Several studies on pedigrees and coefficients of parentage on cotton cultivars released in USA show that diversity was declining due to the frequent use of a few parents (Bowman et al. 1996, 2003; Van Esbroeck et al. 1998, 1999; Bowman and Gutierrez 2003). The use of the exotic *G. hirsutum* germplasm collection is one important approach for obtaining new alleles for cotton improvement. These primitive accessions of cotton have been shown to have useful traits (Percival 1987; McCarty et al. 1995, 1998a, b, 2003, 2004a, b); however, direct use of these accessions has been limited because most are photoperiod sensitive and require short days to initiate flowers and produce harvestable fruits (cotton bolls). Thus, incorporating day-neutral genes into the primitive accessions through a backcross breeding program is being used, so that they will flower in cotton breeding nurseries (McCarty et al. 1979; McCarty and Jenkins 1993). Advanced day-neutral lines from a number of backcrosses to different accession parents have been evaluated for several agronomic and fiber traits (McCarty et al. 1995, 1998a, b; Swindle 1993). The use of derived primitive accessions in a cotton breeding program has enriched genetic resources and can avoid the incompatibility between the genomes which usually occurs in interspecific crosses.

Swindle (1993) analyzed the yield and fiber data set obtained for day-neutral primitive cottons after zero to four backcross cycles (F_6, BC_1F_6, BC_2F_6, BC_3F_6, BC_4F_6) to the primitive cotton, from each of four original photoperiod sensitive accessions, T78, T174, T326, and T1149. These 20 day-neutral populations were crossed with each of four commercial cultivars, DES119; Deltapine 50 (DP50), Stoneville 453(ST453), and Coker 315(C315). The 80 F_2 hybrids, 20 day-neutral parental lines, and four cultivars were evaluated for yield, yield components, and fiber traits in two environments, at Mississippi State, MS in 1989. Swindle (1993) evaluated genetic relationships between different numbers of backcrosses of these accessions, for agronomic and fiber traits in F_2 hybrid form, and found there were no consistent patterns associated with the number of backcrosses. Zhong (2001) and Zhong et al. (2002) used AFLP markers and further revealed the genetic relationship among the four commercial cultivars, 20 day-neutral parental populations and their four original primitive accessions, T78, T174, T326, and T1149 used in Swindle's study (1993). The results by Zhong et al. (2002) also showed that recovery rate of AFLP markers from the primitive accessions had

no consistent patterns with the number of backcrosses. Swindle (1993) and Zhong et al. (2002) evaluated this germplasm at the phenotypic and molecular levels, respectively, and reported similar results; however, the associations between quantitative traits and AFLP markers in this germplasm were not evaluated. In our current study, we focused on identifying AFLP markers associations with agronomic and fiber traits using the combined data from Swindle (1993) and Zhong (2001). This study should provide important information on MAS to improve efficiency in introgressing alleles from exotic accessions into cotton cultivar development.

Material and methods

Materials

Four photoperiod sensitive accessions of cotton were crossed with the day-neutral flowering donor parent 'Deltapine 16' (DP16). The accessions were T78 (PI 549140) race latifolium, T174 (PI 163647) race latifolium, T326 (PI 165326), and T1149 (PI 529966). T326 has the appearance of race palmeri and T1149 the race latifolium. Descriptive data for these accessions were provided by Percival (1987). Twenty day-neutral (DN) populations (F_6, BC_1F_6, BC_2F_6, BC_3F_6, and BC_4F_6, for each of the four accessions) were used (Table 1). The development of these DN populations was as follows. The first cycle (F_1) in each accession was obtained from a cross between the photoperiod sensitive accession and 'Deltapine 16'. Cycle two ($BC1F_1$) in each accession was the first backcross to the photoperiod sensitive parent using F_3 plants from one F_2 day neutral plant. This procedure continued until cycle 5 ($BC4F_1$). The first cross and subsequent backcrosses were increased to the F_5 generation by natural self pollination and were selfed in the winter nursery to provide seed for the F_6 generations planted for field data. The detailed description for these DN populations was provided by Swindle (1993). Four cultivars, 'DES 119', 'Deltapine 50' (DP50), 'Stoneville 453' (ST453), and 'Coker 315' (C315), which had been used as parents in further crosses, were also evaluated.

Table 1 Twenty day-neutral lines and four cultivars (modified from Swindle, 1993)

Entry no.[a]	Generation	Accession parent
Group T78		
1	F_6	T-0078
2	BC_1F_6	T-0078
3	BC_2F_6	T-0078
4	BC_3F_6	T-0078
5	BC_4F_6	T-0078
Group T174		
6	F_6	T-0174
7	BC_1F_6	T-0174
8	BC_2F_6	T-0174
9	BC_3F_6	T-0174
10	BC_4F_6	T-0174
Group T326		
11	F_6	T-0326
12	BC_1F_6	T-0326
13	BC_2F_6	T-0326
14	BC_3F_6	T-0326
15	BC_4F_6	T-0326
Group T1149		
16	F_6	T-1149
17	BC_1F_6	T-1149
18	BC_2F_6	T-1149
19	BC_3F_6	T-1149
20	BC_4F_6	T-1149
Group cultivar		
21	DES119	–
22	Deltapine 50	–
23	Stoneville 453	–
24	Coker 315	–

[a] Entries 1–20 are day-neutral lines developed from backcross programs with each of four primitive accessions, T-78, T-174, T-326, and T-1149, respectively. Entries 21–24 are four commercial cultivars

Experiment

In the original study that provided the agronomic and fiber data, 20 DN populations and four cultivars were planted in two locations at Mississippi State, MS in 1989 (Swindle 1993). A randomized complete block design with four replications was applied for each location. Soil type for location one was a Leeper silty clay loam (fine-loamy, montmorillonitic, nonacid, thermic Vertic Haplaquept) and location two was a Marietta sandy clay loam (fine-loamy, siliceous, thermic Fluvaquentic Eutrochrept). Planting dates were April 26 and 27 at the two locations, respectively. Plots were single rows, 12.3 m long with spacing of approximate 1 m between rows. Standard cultural and insect control practices were followed on these plots.

Prior to machine harvest, a 50-boll sample was hand picked from each plot for boll weight and lint percentage determinations. The lint samples were sent to a commercial laboratory (StarLab, Knoxville, TN) for determining 2.5% fiber span length, micronaire, elongation, and fiber strength. Seed yield and lint yield were converted from plot weight and expressed as kg/ha. In this manuscript, however, agronomic and fiber traits over the two environments for these lines were used.

In a recent study that provided the DNA samples for AFLP analysis, seeds from the 20 DN lines with their four original photoperiodic parental lines and the four cultivars, DES 119, DP 50, ST 453, and Coker 315, were planted in the field in 1999 (Zhong 2001; Zhong et al. 2002).

AFLP analysis

Eight *Eco*RI and eight *Mse*I primers in all 64 possible *Eco*RI–*Mse*I combinations were used (Table 2) (Zhong 2001). Forty-three primer pairs that gave repeatable results for the DNA amplification were chosen to use in the AFLP analyses for the four original primitive accessions and the 24 genotypes (20 DN and 4 cultivar parents) in Swindle's study. The markers had a band size range from 90 bps to 450 bps and only peak heights greater than 35 were scored as present. All AFLP fragments were scored as dominant markers. Each AFLP marker was scored as 1 for presence and 0 for absence. AFLP markers are designated with the name of the two primers, e.g., E1M2, used to amplify the DNA, followed by the estimated band size (bp). For example, a polymorphic AFLP marker with the combination of *Eco*RI E1 and *Mse*I M2 with band size 106 is designated as E1M2-106.

Statistical analyses

We detected 119 AFLP markers that showed polymorphism among the 20 DN populations and four cultivars. Multiple comparisons among the 24 genotypes for each trait were conducted. Mean values for agronomic and fiber traits over two environments were used to detect the coefficients of correlations between AFLP markers and quantitative traits. AFLP markers significantly associated ($\alpha = 0.05$) with quantitative traits were used in multiple linear regression analyses for all traits. Considering that some AFLP markers may be correlated, the stepwise regression procedure was applied for marker selection in the linear regression analyses and the cumulative coefficients of determination were obtained. All data analyses were run by SAS program (SAS Institute Inc. 2001).

Results

Mean values of 24 genotypes for agronomic and fiber traits

On average, cultivars had higher lint yield than all four groups of DN populations (Table 3). Group T78 was the lowest in lint yield among all five groups. All four DN groups yielded less than 800 kg/ha lint. Cultivars had the largest boll size among five groups. Group T-0174 had the largest boll size among four DN groups. On average,

Table 2 Primer pairs selected to use in AFLP marker analysis (Zhong et al. 2001)

*Eco*RI	*Mse*I							
	CAA (M1)	CAC (M2)	CAG (M3)	CAT (M4)	CTA (M5)	CTC (M6)	CTG (M7)	CTT (M8)
AAC (E1)	X[a]	X	X	X	X		X	
AAG (E2)		X						
ACA (E3)	X	X	X	X	X	X		X
ACC (E4)			X	X	X	X	X	X
ACG (E5)		X	X			X		
ACT (E6)	X	X	X	X	X	X	X	X
AGC (E7)	X		X	X		X		
AGG (E8)	X	X	X	X	X	X	X	X

[a] X indicates the combination was used

cultivars had the highest lint percentage among five groups, greater than 40%; whereas, the group T-0078 was the lowest, less than 33%. Groups T-0174 and T-0326 had higher micronaire values than the other three groups. Cultivars had the longest fibers and the group T-0078 had the shortest fibers among five groups. Groups T-0174 and T-0326 had lower fiber elongation than the other three groups. Group T-0174 was the strongest and groups T78 and T1149 were the weakest for fibers among the five groups.

The successive backcrosses to the recurrent primitive accession did not show any trends for the agronomic and fiber traits measured (Table 3). McCarty et al. (1998a, b) also observed similar results when they examined several different backcross cycles of primitive accessions for effects on agronomic and fiber traits. Large differences for lint yield between the four cultivars and the 20 DN populations were observed. Numerically, all four cultivars yielded more than 1095 kg lint ha^{-1}, while all DN populations yielded lint less than 850 kg ha^{-1}. In general, cultivars had greater boll weight than DN populations. DP50, ST453, and C315 had boll weights significantly greater than 5 g and only two DN populations (6 and 10) both from T174 had boll weight greater than 5 g. Five DN populations had boll weight significantly less than 5 g. DES119 and ST453 had lint fractions significantly greater than 40%. All cultivars had greater lint percentage than DN populations except populations 11, 12, and 16, which did not significantly differ from DP50. Micronaire ranged from 4.22 to 4.70 among cultivars. Micronaire for DN lines ranged from 3.96 to 5.20. On the average, the cultivars had greater 2.5% span length than the DN populations. All DN populations except 13 and 15 had

Table 3 Means values for agronomic and fiber traits for 24 cotton lines over two locations

Entry no.[a]	LY kg ha^{-1}	BW g	LP %	MIC	SL25 Mm	E1 %	T1 KNm kg^{-1}
1	490	4.75	31.29	3.96	27.05	8.59	201
2	469	4.90	32.28	4.55	25.65	8.66	193
3	719	4.79	33.53	4.78	26.04	8.19	202
4	369	4.05	30.53	4.05	25.62	8.94	190
5	488	5.30	34.58	5.20	25.88	8.69	194
6	713	5.42	34.94	5.03	26.89	8.75	213
7	837	5.08	35.44	4.39	27.34	8.06	200
8	753	5.10	34.80	4.61	27.11	8.22	197
9	737	4.78	35.42	4.89	27.94	6.97	216
10	618	5.36	34.11	5.13	26.29	7.44	208
11	724	3.96	38.29	4.81	26.70	8.66	202
12	850	5.08	37.40	4.55	27.56	8.13	204
13	746	4.61	35.99	4.50	28.13	7.56	211
14	561	4.90	32.84	4.81	26.89	7.75	206
15	578	4.80	35.21	4.98	28.26	8.03	209
16	848	4.71	37.06	4.53	26.23	8.56	197
17	525	4.84	33.73	4.63	26.77	9.16	200
18	747	5.15	34.89	4.89	26.38	8.09	203
19	737	5.08	36.32	4.49	27.37	8.38	192
20	743	5.13	34.25	4.45	26.38	9.66	181
21	1157	5.22	41.36	4.70	28.96	9.06	205
22	1095	5.59	38.16	4.48	29.16	9.24	193
23	1275	5.84	43.37	4.66	28.65	8.73	194
24	1096	5.70	41.12	4.22	30.44	7.55	221
LSD0.05$^{\Delta}$	146	0.34	1.24	0.23	0.60	0.59	8
Group							
T-0078	507	4.76	32.44	4.51	26.05	8.61	196
T-0174	731	5.15	34.94	4.81	27.11	7.89	207
T-0326	692	4.67	35.95	4.73	27.51	8.03	206
T-1149	720	4.98	35.25	4.60	26.63	8.77	195
Cultivar	1156	5.59	41.00	4.52	29.31	8.65	203
LSD0.05$^{\theta}$	65	0.15	0.55	0.10	0.27	0.26	4

LY = lint yield; BW = boll weight; LP = lint percentage; MIC-micronaire; SL2.5 = 2.5% span length; E1 = elongation; and T1 = fiber strength

[a] See Table 1 for entry designation

Δ is the LSD value for 24 entries and θ the LSD value for groups

2.5% span length less than 28 mm. C315 had fiber strength of 220.8 kNm kg^{-1}. DN populations 6, 9, 13, and 15 had fibers with strength significantly greater than 200 kNm kg^{-1}. In summary, cultivars produced more lint yield, heavier bolls, higher lint percentage, and longer fibers than most DN population and some DN population had stronger fiber than three of the cultivars.

Correlations between AFLP markers and agronomic and fiber traits

Correlation analyses between AFLP markers and quantitative traits were conducted and Pearson's coefficients of correlations that were significant at the 0.05 probability were calculated. Among the 119 AFLP markers there were 50 that were significantly associated positively or negatively with lint yield, 23 with boll weight, 45 with lint percentage, five with micronaire, 43 with 2.5% span length, 11 with elongation, and 12 with fiber strength. There were 11 markers associated with lint yield, five with boll weight, 13 with lint percentage, 12 with 2.5% span length, and one with fiber strength in which the correlation coefficients were equal to or greater than 0.60 or equal to or less than –0.60. Some AFLP markers had strong associations with more than one agronomic and fiber traits. For example, marker E1M1-106 had positive correlations of 0.82 with lint yield, 0.60 with boll weight, 0.78 with lint percentage, and 0.78 with 2.5% span length. Thus, cotton genotypes with marker E1M1-106 present are expected to have higher lint yield and lint percentage, heavier bolls, and greater 2.5% span length. Marker E1M3-168 had strong negative correlations with lint yield, lint percentage, and fiber length. Thus, genotypes with marker E1M3-168 present are expected to have low yield and lint percentage, small bolls, and shorter fiber 2.5% span lengths.

In summary, a number of AFLP markers were significantly associated with lint yield, lint percentage, boll weight, and fiber strength in this study. The results also suggested that some AFLP markers may be closely linked because several markers were highly associated with the same traits. AFLP markers having significant associations with micronaire, elongation, and fiber strength were fewer in number and appeared not to be associated with yield.

Multiple linear regression of agronomic and fiber traits on AFLP markers

Only markers significantly correlated with a specific quantitative trait were used for multiple linear regression analysis for that specific trait. Four AFLP makers E1M1-106, E1M4-153, E8M8-229, and E8M5-231, were selected by the linear regression model for lint yield (Table 4). Marker E1M1-106 was associated with 67% of the phenotypic variation for lint yield, while markers E1M1-106 and E1M4-153 together were associated with 85% of the total variation for lint yield. Marker E7M6-179 was the most important for boll weight and was associated with 43% of the total variation for this trait. Three additional markers, E3M6-288, E3M5-183, and E1M2-139, were also selected in the multiple linear regression analysis and the four markers together were associated with 83% of the total variation for boll weight. Markers E6M3-266, M3M6-219, and E1M3-168 were associated with 86% of the variation for lint percentage. Marker E6M1-314 was the only marker selected by the regression model for micronaire, and was associated with 22% of its total variation. Markers E1M1-106 and E1M4-203 together were associated with 76% of the total variation for 2.5% span length. AFLP markers E3M8-201, E1M3-335, and E3M5-137 together were associated with 63% of the total variation in fiber strength, and E5M6-260, E1M4-421, and E1M4-335 with 55% for elongation.

Comparing the distribution of the selected AFLP markers among the 24 genotypes, we found that the results (Table 4) were in a good agreement with observed AFLP and phenotypic data (Tables 3 and 5). For example, four cultivars with marker E1M1-106 present produced higher lint yields and longer fibers while 20 DN populations with this marker absent had lower lint yield and shorter fibers (Tables 3 and 5). Marker E1M3-168 was absent in DN population 16 and the four cultivars which had higher lint percentage; whereas this marker was present in all other DN populations and they had lower lint percentage. Marker E1M3-168 was associated with

Table 4 Selected AFLP markers in multiple linear regression analyses for agronomic and fiber traits

	Lint yield			
Marker[a]	E1M1-106	E1M4-153	E8M8-229	E8M5-231
R^2 [b]	0.67	0.85	0.89	0.93
	Boll weight			
Marker	E7M6-179	E3M6-288	E3M5-183	E1M2-139
R^2	0.43	0.65	0.74	0.83
	Lint percentage			
Marker	E6M3-266	E3M6-219	E1M3-168	E3M5-405
R^2	0.65	0.78	0.86	0.90
	2.5% span length			
Marker	E1M1-106	E1M4-203	E3M5-137	E3M6-236
R^2	0.60	0.76	0.81	0.85
	Elongation			
Marker	E5M6-260	E1M4-421	E1M4-335	
R^2	0.32	0.44	0.55	
	Fiber strength			
Marker	E3M8-201	E3M5-137	E1M4-335	
R^2	0.40	0.53	0.63	
	Micronaire			
Marker	E6M1-314			
R^2	0.22			

[a] Cumulative markers in multiple linear regression model

[b] Cumulative coefficient of determination in multiple linear regression models

low lint percentage and marker E6M3-266 was associated with high lint percentage (Tables 3 and 5). The results suggested that two positive genetic effects for lint percentage were associated with markers E1M3-168 and E6M3-266 in the four cultivars, one positive genetic effect for lint percentage was associated with marker E1M3-168 in DN population 16 and one positive genetic effect associated with marker E6M3-266 was associated with lint percentage in DN populations 11 and 12. Similar results for markers E1M1-106 and E1M4-153 associated with lint yield were also observed.

In summary, we detected a few AFLP makers that accounted for 83% or more of the total variance for all agronomic traits measured and 2.5% fiber span length, respectively, indicating that these AFLP markers could be used for MAS in the improvement of lint yield, boll weight, lint percentage, and 2.5% span length in crosses with these DN populations. A few AFLP markers could also be used to improve elongation and fiber strength via MAS selection.

Discussion

The genotypes for many quantitative traits are not easily visualized. DNA markers provide genetic information to help identify the loci that control a specific quantitative trait. The process of mapping this type of locus requires a mapping population, DNA markers, and phenotypic data. With specific statistical methods the QTLs for quantitative traits can be detected.

In many QTL mapping reports in cotton, major QTLs for yield or fiber quality have not been identified (Shappley et al. 1998b; Ulloa and Meredith 2000; Kohel et al. 2001; Zhang et al. 2001, 2003; Guo et al. 2003; Zuo et al. 2000; Ulloa et al. 2002, 2005; Paterson et al. 2003; Lacape et al. 2005; Zhang et al. 2005). The possible reasons include (1) the selected parental lines are not genetically diverse, (2) the number of genes controlling that quantitative trait is large, or (3) the number of DNA markers detected was very limited and thus strong associations with quantitative traits could not be observed. In this study, we found that four cultivars and 20 DN populations were different in terms of DNA markers and agronomic and fiber traits. A few AFLP markers were associated with a large amount of variation in all three agronomic traits and for fiber length. Even for micronaire, one marker was associated with 20% of the phenotypic variation, which is higher than has been reported. Traditionally, scientists believed that the number of

Table 5 Distributions of markers selected in regression models for agronomic and fiber traits among DP16, 24 entries and their original accessions

Entry	Marker code																			
	E1M1 -106	E1M2- 139	E1M3 - 168	E1M4 -153	E1M4 -203	E1M4 -335	E1M4 -421	E3M5 -137	E3M5 -183	E3M5 -405	E3M6 -219	E3M6 -236	E3M6 -288	E3M8 -201	E5M6 -260	E6M1 -314	E6M3 -266	E7M6 -179	E8M5 -231	E8M8 -229
T-0078	0[a]	0	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	1
1	0	1	1	1	0	0	0	0	1	1	1	0	1	1	0	0	0	1	1	1
2	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	1	1	1
3	0	1	1	0	1	0	0	0	1	1	1	0	1	1	0	0	0	1	0	1
4	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	1	0	1
5	0	1	1	1	1	0	0	0	1	1	0	1	1	1	0	0	0	0	0	1
T-0174	0	1	1	0	0	0	1	0	1	1	0	0	0	0	1	1	0	0	0	1
6	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	1
7	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	1	1
8	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	1	1
9	0	1	1	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	1	1
10	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1
T-0326	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1
11	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	1	1	1	1	1
12	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	1	1	1	1
13	0	1	1	0	0	0	0	0	1	1	0	0	1	0	1	0	0	1	1	1
14	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	0	1
15	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	1
T-1149	0	1	0	0	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0
16	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1	1
17	0	1	1	0	0	0	0	0	1	1	0	1	0	1	0	1	0	1	1	0
18	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1	1
19	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1
20	0	1	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	1
DP16	1	1	1	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	1
21	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0
22	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0
23	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	0
24	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	1	0
	LY^+ SL^+	BW^+	LP^-	LY^-	SL^-	$E1^+$ $T1^+$	EL^-	SL^+ $T1^+$	BW^+	LP^-	LP^-	SL^-	BW^-	$T1^-$	$E1^-$	MIC^+	LP^+	BW^-	LY^+	LY^-

LY=lint yield; BW=boll weight; LP=lint percentage; MIC-micronaire; SL=2.5% span length; E1=elongation; and T1=fiber strength

Superscripts + and – following each trait mean positive and negative correlation between an AFLP marker and the quantitative trait

[a] 0 indicates marker absent: 1 indicates marker present

genes that control yield is large and the genetic effect from each gene is small; however our report showed that selection of major genes controlling cotton yield, lint percentage, and fiber length are possible when ideal experimental materials are used. Therefore, these exotic cotton lines can be used to develop several mapping populations to identify the major QTLs for agronomic and fiber traits of importance using different DNA markers like SSR and RFLP. On the other hand, the results obtained from 24 lines (this study) might be limited, thus more lines should be screened before the further use of some reported AFLP markers as a tool for molecular breeding.

A large number of AFLP markers were found to be strongly associated with yield, boll weight, and lint percentage. However, only a few AFLP markers were selected in our linear regression analyses and these selected AFLP markers were responsible for the majority of the phenotypic variation for these important traits. Only a few markers were associated with micronaire, elongation, and fiber strength in this study. Cotton chromosome substitution lines can be used to assign these markers to specific chromosomes (Saha et al. 2004).

Our data analysis was based on AFLP markers and phenotypic data in a bulked population which at F_6 could still be segregating. Thus, a bulked population with at least one plant having an AFLP present could result in scoring the presence of this AFLP marker in this population since the bulked DNA sample for each population was used. However, the day-neutral lines were observed in field plots and appeared to be uniform within plot and across replications, indicating evidence of these DN lines being highly homozygous. If we assume that an AFLP marker is associated with an improved trait and the number of plants with the presence of this AFLP marker varies greatly among different populations, this scenario should result in a weak association between this AFLP marker and the trait. Thus, when a strong AFLP marker association with a trait is detected in a population, this indicates that the majority of the individual plants in a population probably have this AFLP marker. For example, 17 out of 20 DN populations had low lint percentage. AFLP marker E6M3-266 was detected to have a strong and positive association with lint percentage and this AFLP marker was present in four cultivars and in DN populations 11 and 12, which had higher lint percentage. If only a few plants in these two DN populations had this marker present, then high lint percentage would be unlikely because this trait was determined by bulked boll samples. Thus, our use of marker association analysis via stepwise multiple regression analysis seems to be both useful and supported by the data.

Although AFLP markers have been reported for linkage mapping in several papers (Lacape et al. 2003, 2005; Mei et al. 2004; Zhang et al. 2005), the use of AFLP markers of interest in breeding programs still may be difficult due to the technical complexity. Thus, the conversion of the AFLP markers of interest into breeder-friendly markers is necessary for the practical use in future breeding programs.

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